

## ***Interactive comment on “Fluorescence calibration method for single-particle aerosol fluorescence instruments” by Ellis Shipley Robinson et al.***

**Ellis Shipley Robinson et al.**

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Response to RC1 - amt2016-331

We would like to thank the reviewer for their time looking over our manuscript. This feedback really truly very helpful, both in it being thorough and thoughtful. Thank you very much for reading the paper. We have organized our responses to the reviews by using the same numbering as the initial review, and responding below the reviewer's comments.

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With the growth of biological aerosol research in the last decade, commercially avail-

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able instruments are now available that utilize fluorescence techniques to quantify bio-aerosol concentrations and properties. This paper provides a framework for calibrating such fluorescence instruments, specifically to provide a method to normalize the fluorescence intensity response over time and between different instruments with different operational settings. This work is well done, well presented, and is a welcomed addition to research in this topic area. I do have some reservations as to the application of normalized fluorescence intensities to ambient data and suggest revising the manuscript to address these hesitations. Otherwise, I support publishing the paper after the authors address the following minor comments:

1. Line-80: Please state which requirements were not fulfilled by NADH and naphthalene here. Were any other materials considered and not used? This information might be useful to other groups seeking additional calibration candidates.

-As stated on line 81 "Results from all materials tested are presented in Section 3," so we are not omitting any useful information. A sentence describing the shortcomings of each NADH and naphthalene has been added here to address this concern, while the full details are in section 3.3. This section previously was labeled "Other materials," but we have changed the title to "Failed calibrants: NADH and naphthalene" to set apart these materials from quinine and tryptophan.

2. Section 2.1: One aspect of WBS operation not discussed is timing. I suggest adding a short paragraph summarizing how the instrument timing was set, so this procedure can be used consistently by the community.

-We have added a short paragraph summarizing how we set the timing. "The timing of the firing of each flash lamp was set using the optimization function in the WBS acquisition software while sampling monodisperse fluorescent particles, typically FPLs though the size-selected calibration particles presented here work as well. The timing optimization program scans through a wide range of delay times for the lamps for a given fluorescent channel following triggering (detecting the scattered light pulse). The

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software simultaneously averages fluorescent signals. The delay time corresponding to the maximum average fluorescent signal determines the optimal flash lamp timing. Flash lamp timing was periodically determined for each fluorescent channel, but did not vary over the course of these measurements."

3. Line-118: It is interesting that peak height varies as a function of flow rate and I certainly agree that calibrations should be done at an appropriate flow rate. Since the data in Figure 1 seem to asymptote toward a constant response at higher flow rates, would you suggest that users operate at a flow rate  $> 4$  LPM so peak height is less sensitive to small variations in flow rate? Users would have to accept limitations of decreased counting efficiency for high concentrations at these higher flow rates.

-We appreciate that the peak height for the data in Figure 1 do converge for the highest flow rates presented. However, what we think this really means is that at higher flow rates the instrument's ability to resolve the true scattering peak height is getting worse and worse. So, while at low flow rates we are more sensitive to fluctuations in flow, the instrument is better able to resolve peak heights. Luckily, we don't expect large fluctuations in flow through the instrument in most sampling applications, as the sample and sheath flowrates are controlled by precision flow controllers with stated accuracies within 1% of the reading. So we don't expect this issue to be a real concern, and in fact have reason to operate at lower flow rates when possible. Our goal in presenting Figure 1 was to illustrate an issue that exists for the WIBS-4A model, and emphasize that fluorescence and size calibrations are only valid for a given flow configuration. The limitations of a given sampling situation may necessitate operating the WIBS at different flow rates, which would require different calibrations.

4. Line-162: What model DMA was used here, and is it able to size select particles greater than 1 micron diameter?

-As stated in the original text, the DMA used here is custom-built, and yes it can select particles greater than 1  $\mu\text{m}$ . There is no previous instrument paper to cite for the

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NOAA DMA, though we have added to the text that the NOAA DMA column is longer than e.g. TSI 3081, which gives it the ability to select larger particles. See P6 L171 of the updated text for a mention of this: "It should be noted that the custom-built DMA has a longer column than some commercially-available versions (e.g. TSI 3081), and so is more easily able to select larger size particles."

5. Line-438: Larger, philosophical issue. Expressing fluorescence intensity as T- and Q-units is certainly a clean and easy way of comparing the output of different instruments to each other. But, there is a risk of largely over-simplifying the interpretation of ambient results where many complex factors govern fluorescence intensity. This is noted explicitly in the Pohlker review of bioaerosol auto-fluorescence, "However, fluorescence intensity is a complex function of various parameters such as concentration, extinction coefficient at  $\lambda_{ex}$  and quantum yield at  $\lambda_{em}$  as well as influences by the molecular environment. Accordingly, only semi-quantitative comparison of intensity levels is possible based on the presented results." For example, two ambient populations could result in the same Q-unit fluorescence and have very different actual amounts of fluorescent material because of the properties listed above. Interpreted results that showed similar Q-unit fluorescence intensity would not be at all accurate in this case. By advocating the use of T- and Q-units, are we over-simplifying these systems and risking erroneous interpretation? There is no doubt that using this method to ensure instruments are operating similarly is very beneficial, but I question the application to ambient analyses. I suggest adding a caution to users wishing to apply T- and Q-units for ambient applications, potentially including a review of the large range of quantum yields for fluorescent material, and removing this recommendation from the conclusions (Line-453).

-We completely agree with the reviewer on most of the points made in this comment. For example, "two ambient populations could result in the same Q-unit fluorescence and have very different actual amounts of fluorescent material." This is absolutely correct, for the reasons cited above in Pohlker, et al. However, we do not quite understand

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sentence that follows, "Interpreted results that showed similar Q-unit fluorescence intensity would not be at all accurate in this case." What is being referred to that is not accurate? The ultimate goal of this calibration method is not to quantify the amount of fluorophore mass in e.g. ambient particles by measuring their fluorescence with the WIBS. Rather, the goal in using this calibration method is to create a scale that is not completely arbitrary, and thus can be used over time and across instruments. Using "Q-units," or something similar, aerosol populations from different datasets could be compared to each other, and it would be possible to say "population A in study 1 has the same fluorescence as population B in study 2." That is the step forward we hope that this paper makes possible. It would be an erroneous leap to go one step further, as the reviewer points out, to say that the amount of fluorescent mass in population A and population B are the same, but that is not what our paper is advocating for. The last paragraph of section 3.5 makes this clear, where we relate the fluorescence from Blue 1 micron FPSLs to the fluorescence of a mass of quinine under the operating conditions of our instrument. But we are not saying we have any knowledge of the mass of the actual fluorophores in the PSL particles.

Pöhlker, C., J. A. Huffman, and U. Pöschl. "Auto-fluorescence of atmospheric bioaerosols—fluorescent biomolecules and potential interferences." *Atmospheric Measurement Techniques* 5.1 (2012): 37-71.

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